from the early methionine (*Em*) gene from wheat (Marcotte et al., *Plant Cell* 1:976-979 (1989); the cauliflower mosaic virus (CaMV) 35S promoter (Odell et al., *Nature* 313:810-812 (1985); and the nopaline synthase (nos) promoter (Sanders et al., *Nucl. Acids Res.* 15(4):1543-58 (1987). Tissue-specific plant promoters or plant promoters responsive to chemical, hormone, heat or light treatments may be used. Additionally, the gene of interest may be expressed under the control of its endogenous promoter to ensure proper regulation.

[0120] The process of transformation requires plant cells that are competent and that are either embryogenic or organogenic. The plant cells to be transformed are then co-cultivated with *Agrobacterium* containing an engineered T-DNA vector system for 1-5 days. Following the co-cultivation period, the cells are incubated with the antibiotic against which the selectable marker confers resistance, and transformed lines are selected for further cultivation. The use of *Agrobacterium* mediated transfer in woody trees is described in Loopstra et al., *Plant Molecular Biology* 15:1-9 (1990), Gallardo et al., *Planta* 210:19-26 (1999) and Wenck et al., *Plant Molecular Biology* 39:407-419 (1999), each of which is hereby incorporated by reference.

<u>Direct Gene Transfer by Particle Bombardment</u>

[0121] Direct gene transfer by particle bombardment provides another method for transforming plant tissue. This method can be especially useful when plant species are recalcitrant to transformation by other means. In this technique a particle, or microprojectile, coated with DNA is shot through the physical barriers of the cell. Particle bombardment can be used to introduce DNA into any target tissue that is penetrable by DNA coated particles, but for stable transformation, it is imperative that

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regenerable cells be used. Typically, the particles are made of gold or tungsten. The particles are coated with DNA using either $CaCl^2$ or ethanol precipitation methods which are commonly known in the art.

[0122] DNA coated particles are shot out of a particle gun. A suitable particle gun can be purchased from Bio-Rad Laboratories (Hercules, CA). Particle penetration is controlled by varying parameters such as the intensity of the explosive burst, the size of the particles, or the distance particles must travel to reach the target tissue.

[0123] The DNA used for coating the particles should comprise an expression cassette suitable for driving the expression of the gene of interest. Minimally this will comprise a promoter operably linked to the gene of interest. As with *Agrobacterium* mediated transformation. Suitable promoters include, but are not limted to, the the abscisic acid (ABA)-inducible *Em* promoter from wheat (Marcotte et al., *Plant Cell* 1:976-979 (1989), the CaMV35S promoter (Odell et al., *Nature* 313:810-812 (1985), and the NOS:promoter (Sanders et ., *Nucl. Acids Res*. 15(4):1543-58 (1987).

[0124] Methods for performing direct gene transfer by particle bombardment are disclosed in U.S. Patent 5,990,387 to Tomes et al. Additionally, Ellis et al. describe the successful use of direct gene transfer to white spruce and larch trees in *Bio/Technology* 11, 84-89 (1993).

[0125] Researchers skilled in the area of DNA or gene transformation will recognize that additional procedures, or combination of procedures, may be useful for the successful tranformation of genetic stock.

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Antisense Expression

[0126] The cDNAs of the invention may be expressed in such a way as to produce either sense or antisense RNA. Antisense RNA is RNA that has a sequence which is the reverse complement of the mRNA (sense RNA) encoded by a gene. A vector that will drive the expression of antisense RNA is one in which the cDNA is placed in "reverse orientation" with respect to the promoter such that the non-coding strand (rather than the coding strand) is transcribed. The expression of antisense RNA can be used to down-modulate the expression of the protein encoded by the mRNA to which the antisense RNA is complementary. This phenomenon is also known as "antisense suppression." It is believed that down-regulation of protein expression following antisense RNA is caused by the binding of the antisense RNA to the endogenous mRNA molecule to which it is complementary, thereby inhibiting or preventing translation of the endogenous mRNA.

[0127] The antisense RNA expressed need not be the full-length cDNA and need not be exactly homologous to the target mRNA. Generally, however, where the introduced sequence is of shorter length, a higher degree of homology to the endogenous mRNA will be needed for effective antisense suppression. Preferably, the introduced antisense sequence in the vector will be at least 30 nucleotides in length, and improved antisense suppression will typically be observed as the length of the antisense sequence increases. The length of the antisense sequence in the vector may be greater than 100 nucleotides. Vectors producing antisense RNA's could be used to make transgenic plants, as described above, in situations when desirable tree

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